REMARKS/ARGUMENTS

Claim Status

Claims 3-6, 10, 25-26 and 40 have been canceled without prejudice. Claims 1, 8-9, 11, 12, 17, 27 and 41 have been amended. Claims 29-39 have been withdrawn.

Support for amendment of claim 1 to recite an iron hydrogenase can be found, for example, in original claim 3.

Support for amendment of claim 1 to recite "wherein at least one amino acid from the segment $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ (SEQ ID NO:185) and at least one amino acid from the segment ADX^8TIX^9EE (SEQ ID NO:186) are both substituted by a different amino acid in the iron-hydrogenase to generate the mutagenized nucleic acid sequence" can be found, for example, in claim 40. Additional support for this element can be found in the specification at page 22, paragraph 91, through page 25, paragraph 95. This section of the specification discloses, among other things, a gene reassembly method of producing mutagenized iron hydrogenase sequences involving simultaneous substitution of residues in both the $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ segment and the ADX^8TIX^9EE segment. Paragraph 94 on page 24 specifically discloses the gene reassembly reaction disclosed in Figures 13-15. Further support for substituting both segments in the same gene reassembly reaction can be found, for example, in Figures 16 and 17, which disclose variants of the $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ and ADX^8TIX^9EE segments and a diagram of the annealing positions of nucleic acids encoding portions of the $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ and ADX^8TIX^9EE segments, respectively. Figure 17 discloses, among other things, an annealing reaction in which the $X^1X^2X^3FX^4X^5X^6GGV$ portion and the $MEAAX^7R$ portion of the $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ segment are encoded by different nucleic acids placed in the same gene reassembly reaction. Portions of these segments of various lengths are also disclosed on page 24, lines 6-7. Additional examples of support for this element of claim 1 are also discussed at length below in response to the 35 U.S.C. § 112, first paragraph rejection.

Support for amendment of claim 1 to recite "wherein the cell is selected from the group consisting of photosynthetic bacteria, bacteria, and green algae" can be found, for example, in paragraphs 49-51.

Support for amendment of claims 8 and 9 can be found, for example, in claim 40. Additional support for amendment of claim 8 can be found, for example, in paragraph 91, lines 6-11 and in paragraph 93, lines 25-27. Additional support for amendment of claim 9 can be found, for example, in paragraph 93, lines 24-34. Additional examples of support for amendment of these claims can be found immediately above as it pertains to claim 1, as well as discussion below in response to the 35 U.S.C. § 112, first paragraph rejection.

Support for amendment of claim 11 to recite nucleic acid molecules that encode "a plurality of" proteins of SEQ ID NOs: 1-112 or segments thereof can be found in Figure 17, which discloses nucleic acid molecules that encode a plurality of segments of proteins from SEQ NOs: 1-112. Specifically, Figure 17 discloses nucleic acid molecules that encode segments of SEQ ID NOs: 2, 23, 26 and 98.

Support for amendment of claim 27 can be found in original claim 3.

Claim 41 has been amended to include SEQ ID NOs.

Election/Restrictions

Applicant affirms election to prosecute Group 26, claims 2-28, as drawn to SEQ ID NO:26.

Objections to the Specification

The hyperlink objected to by the Examiner has been replaced by language referring to the Joint Genome Institute by replacement of paragraph 124.

Sequence Compliance

Applicant has amended paragraphs 10, 29-31 and 91-93 to include sequence identifiers. Applicant submits herewith a new paper and compact disc sequence listing containing additional sequences from the specification and drawings. All of the sequences

contained in the new sequence listing were present in the application as originally filed, and thus the new sequence listing contains no new matter.

Claim Objections

The Examiner has objected to claims 3, 11 and 26 as containing non-elected subject matter. Claims 3 and 26 are cancelled. Claim 11, as currently amended, recites "The method of claim 15, wherein the gene reassembly is performed using nucleic acid molecules that encode a plurality of proteins of SEQ ID NOs: 1-112 or segments thereof." Applicant presumes that the Examiner is basing the objection on the inclusion of "SEQ ID NOs: 1-112 or segments thereof." The invention includes gene reassembly methods of iron hydrogenases in which specific segments of iron hydrogenases are substituted for the purpose of creating novel iron hydrogenases that contain gas channels that differ structurally from those of wild-type iron hydrogenases. The gene reassembly method depicted in Figures 13-14, which is also exemplified in Figure 17, includes the use of a "scaffold" nucleic acid molecule encoding an entire iron hydrogenase protein. Small oligonucleotides encoding amino acid variants are annealed to the scaffold, followed by extension by DNA polymerase (see Figures 13-14, Steps 4-6). The resulting nucleic acids contain sequence from both the scaffold as well as the oligonucleotides. This process is described in detail in paragraphs 94-95. While Applicant has elected SEQ ID NO:26 according to the Examiner's restriction requirement, claim 11 recites a gene reassembly method that includes the use of short oligonucleotides encoding amino acid segments that are derived from SEQ ID NOs: 1-112. In the embodiment of this method depicted in Figure 17, the scaffold nucleic acid is from *C. reinhardtii* (elected SEQ ID NO:26) while short oligonucleotides in the reaction are designed from segments of other iron hydrogenases (SEQ ID NOs: 2, 23 and 98). The mutagenesis reaction depicted in Figure 17 is simply a method of mutagenizing SEQ ID NO:26, which is entirely consistent with the Examiner's restriction requirement and Applicant's election. The fact that some short oligonucleotides used to mutagenized SEQ ID NO:26 in this particular embodiment are derived from other iron hydrogenases such as SEQ ID NOs: 1-112 does not render claim 11 non-elected subject matter.

Thus, Applicant respectfully traverses this objection and requests that the Examiner withdraw the objection.

The Examiner's requested grammatical change to claim 17 is reflected in the amended claim set. Claims 5 and 40 have been canceled. Claim 41 has been amended to include sequence identifiers.

35 U.S.C. § 112, second paragraph

The Examiner has rejected claim 1 as vague and indefinite, specifically asserting that the metes and bounds of a nucleic acid sequence "derived from a first gene" are unclear. The Examiner asks (a) *what steps are involved in the deriving* and (b) *which structural features are indicative of such a mutagenized nucleic acid.*

Applicant's use of the term "first gene" is simply a convention of claim drafting that enables the later introduction of a "second gene" distinct from the first gene in a subsequent dependent claim. The term "mutagenized" is a generic term that describes that status of a nucleic acid that has been altered in sequence from a progenitor molecule. A gene can be mutagenized in any number of ways, including site-directed mutagenesis, random mutagenesis, and gene reassembly methods such as those described in the instant specification; these and others are methods that are extremely familiar to those of skill in the art. A nucleic acid generated by a mutagenesis technique is "derived from" a progenitor nucleic acid. *The steps involved in the deriving are therefore any steps, included those recited above, that alter the sequence of a nucleic acid.* For example, in a site directed mutagenesis PCR reaction in which a mutation is introduced through one of the primers, the PCR product (ie: the mutagenized sequence) contains a sequence difference compared to the template (ie: the progenitor molecule). In other words, the product is "derived from" the template. *The structural features/sequences indicative of such a mutagenized nucleic acid are therefore any sequence differences between the mutagenized sequence and the sequence it was derived from.* A person skilled in the art would understand the meaning of the terminology "a mutagenized nucleic acid sequence derived from a first gene..." The Applicant respectfully requests withdrawal of the rejection of claim 1 as vague and indefinite.

The Examiner has rejected claim 10 as vague and indefinite. Claim 10 is cancelled.

The Examiner has rejected claim 12 as vague and indefinite in that the metes and bounds of the term "functionally interacts" is unclear. Applicant directs the Examiner's attention to paragraph 89, lines 14-15, which reads "'Functionally interact' means that a ferredoxin or other electron donor transfers electrons to the hydrogenase protein." Within the context of claim 12 the recitation of this specifically defined term can be further explained by lines 15-18 of paragraph 89, which read "Preferably the sequence change(s) caused by the mutagenesis of the *C. reinhardtii* iron hydrogenase gene does not disrupt the functional interaction between the protein encoded by the mutagenized *C. reinhardtii* iron hydrogenase gene and ferredoxin or another electron donor." Applicant respectfully requests that the Examiner withdraw the rejection.

35 U.S.C. § 112, first paragraph

The Examiner has rejected claims 40-41 as failing to comply with the written description requirement. The Examiner asserts that the content of these claims is new matter. Specifically, the Examiner asserts that the specification as originally filed does not provide "blazemarks nor direction for the instant methods encompassed" by these claims. Claim 40 has been cancelled, and elements from claim 40 have been incorporated into currently amended claim 1. Claim 41 has been amended for the sole purpose of inserting sequence identifiers.

Applicant directs Examiner's attention to paragraphs 94-95 and Figures 13, 14, 15 and 17, which specifically disclose mutagenesis wherein at least one amino acid from the $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ segment and at least one amino acid from the ADX^8TIX^9EE segment are both substituted in the same reaction. Paragraph 94 discloses mutagenesis of an iron hydrogenase in which a plurality of segments of the gene are substituted by the annealing of oligonucleotides to complementary single stranded scaffold nucleic acid. The gene reassembly method generates novel iron hydrogenases in which multiple segments contain amino acid variations. Figure 17 discloses a specific embodiment of the method disclosed in paragraphs 94-95 and figures 13-15. In Figure 17 an annealing-based mutagenesis method is disclosed in which

(1) an oligo encoding a substitution in the ADX^8TIX^9EE segment anneals to the single stranded scaffold and (2) oligos encoding substitutions in the $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ segment anneal to the same single stranded scaffold. As described in paragraph 94 and as depicted in figures 13-14, after annealing of these oligos to the scaffold, polymerase extends between the oligos, forming a complementary strand that contains substitutions in each of the $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ and ADX^8TIX^9EE segments. This process of making substitutions in two or more segments simultaneously is therefore disclosed in general in Figures 13 and 14 and is explicitly disclosed specifically with respect to the $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ and ADX^8TIX^9EE segments in Figure 17. In addition, after the detailed description of the simultaneous mutagenesis process in paragraph 94 and a summary of the resulting molecules in the first part of paragraph 95, paragraph 95 goes on to state:

> "Although figure 17 depicts one possible arrangement of three diverse oligonucleotides that can be annealed to a single stranded wild type sequence, mixing oligonucleotides corresponding to each of the identified gas channel segments from SEQ ID Nos:124-147 that have C. reinhardtii flanking codons produces a large number of possible combinations of library sequences. Each possible combination corresponds to a different gas channel architecture that can be tested for the ability to allow flow of hydrogen but not oxygen." (lines 25-31 of paragraph 95)

SEQ ID NOs:124-141 are $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ segment variants, while SEQ ID NOs: 142-147 are ADX^8TIX^9EE segment variants. This passage from paragraph 95 as well as Figures 16- 17 provide explicit support for substitution of each of the ADX^8TIX^9EE and $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ motifs in a mutagenesis reaction, as well as explicit support for a method in which more than one nucleic acid encoding at least a portion of the $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ segment and more than one nucleic acid encoding at least a portion of the ADX^8TIX^9EE segment are placed in a gene reassembly reaction. Because explicit support is present in the specification and figures for amended claim 1 and claim 41, Applicant respectfully requests that the Examiner withdraw the new matter rejection.

The Examiner has rejected claims 1-28 and 40-41 on the basis that the claims contain subject matter which was not described in the specification in such a way as to

reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. The general nature of the Examiner's rejection revolves around the idea that the claims are not restricted to any particular cell type or any particular gene, and thus the breadth of the claims is not in accord with the disclosure provided. This general assertion is not correct, as can be seen by examining various parts of the specification. In particular, the Examiner's assertion that the disclosure is restricted to mutagenesis of only an iron hydrogenase and to the use of only green algae cells is not correct.

Paragraph 49 discloses two types of cells that are known to produce hydrogen gas, photosynthetic bacteria (also known as cyanobacteria) and bacteria (ie: non-photosynthetic). All of these organisms are prokaryotic, and as such are very different from *C. reinhardtii* cells or other eukaryotic green algae. Paragraph 49 discloses multiple references that provide examples of prokaryotic species capable of expression of hydrogenase genes, including Appl Microbiol Biotechnol (2001) Dec;57(5-6):751-6 (entitled "Evidence for three distinct hydrogenase activities in *Rhodospirillum rubrum*"); Appl Microbiol Biotechnol (2002) Apr;58(5):618-24 ("Disruption of the uptake hydrogenase gene, but not of the bidirectional hydrogenase gene, leads to enhanced photobiological hydrogen production by the nitrogen-fixing cyanobacterium *Anabaena sp.* PCC 7120"); Biochimie (1986) Jan;68(1):121-32 (entitled "Hydrogenase activities in cyanobacteria", and describing the prokaryotic species *Anacystis nidulans* and *Anabaena 7119*); and J Bacteriol (1985) Apr;162(1):353-60 (entitled "Cloning of hydrogenase genes and fine structure analysis of an operon essential for H_2 metabolism in *Escherichia coli*"). Applicant also directs the Examiner's attention to the fact that in addition to the disclosure in the specification of *E. coli* as a system for hydrogenase expression, persons skilled in the art are currently using *E. coli* for this purpose (see for example King et al., J Bacteriol. 2006 Mar;188(6):2163-72 "Functional studies of [FeFe] hydrogenase maturation in an *Escherichia coli* biosynthetic system"). In addition, as the Examiner is aware, the specification also discloses the use of eukaryotic green algae such as *C. reinhardtii* in, for example, paragraph 52. The specification also discloses the use of the blue-green algae *Anabaena cylindrica* in paragraph 59 (Appl Environ Microbiol 1977 Jan;33(1):123-31).

Applicant also draws the Examiner's attention to the fact that in addition to iron hydrogenases, the specification also discloses numerous other genes involved in hydrogen production pathways, as can be found, for example, in original claim 3 (ferredoxin, catalase, isoamylase, malate dehydrogenase, 14-3-3 protein, enolase, aldolase, ribosomal protein S8, ribosomal protein L17, ribosomal protein S18, ribosomal protein L37, ribosomal protein L12, ribosomal protein S15, nickel-iron hydrogenase, and components of the photosystem I, photosystem II, light harvesting antenna and cytochrome b6-f complexes); and in paragraph 96, lines 3-6 (GenBank accession numbers M24072, AF104630, AF104631, AB050007, X65119 and the genes lhcbm1, lhcbm2, lhcbm3, lhcbm4, lhcbm5, lhcbm6, lhcbm8, lhcbm9, lhcbm11, lhca1, lhca2,lhca3, lhca4, lhca5, lhca6, lhca7, lhca8, lhca9, lhcb4, lhcb5, lhcq, l1818-111818-2, elip1, elip2, elip3, elip4, and elip5).

Applicant has amended claim 1 to recite "wherein the cell is selected from the group consisting of photosynthetic bacteria, bacteria, and green algae". Applicant has also amended claim 1 to recite "an iron-hydrogenase". The amendment of claim 1 should not be interpreted as an admission of any kind that the disclosure and teaching of the application is limited to iron hydrogenases. Applicant respectfully requests the Examiner withdraw the rejection of claims 1-28 and 40-41.

35 U.S.C. § 102

The Examiner has rejected claims 1-4, 12-13, 15-21, 24 and 26 as anticipated by Ahmann. Ahmann discusses a method of DNA shuffling of wild-type cDNAs encoding iron hydrogenases, followed by screening for hydrogen production. For a prior-art reference to anticipate the claimed invention, each element of the claim must be found, expressly or under principles of inherency, in a single prior art reference. *See Diversitech Corp.* v. *Century Step, Inc.*, 7 U.S.P.Q.2d 1315, 1317 (Fed. Cir. 1988).

Claim 1 has been amended to include the element of "providing a mutagenized nucleic acid sequence derived from a first gene that encodes an iron-hydrogenase protein, wherein at least one amino acid from the segment $X^1X^2X^3FX^4X^5X^6GGVMEAAX^7R$ and at least one amino acid from the segment ADX^8TIX^9EE are both substituted by a different amino acid in

the iron-hydrogenase to generate the mutagenized nucleic acid sequence." Nowhere in Ahmann are these elements of the claims or any particular amino acid sequence disclosed.

It is not correct that Ahmann would inherently anticipate the mutagenesis method of amended claim 1. To make out a *prima facie* case of inherency, the Examiner would need to provide factual and technical grounds establishing that the allegedly inherent feature *necessarily* flows from the teachings of the prior art. *See Ex parte Levy,* 17 U.S.P .Q.2d 1461, 1464 (Bd. Pat. App. & Int. 1990). It is well-settled that "[i]nherency . . . may not be established by probabilities or possibilities." *Continental Can Co. USA, Inc.* v. *Monsanto Co.,* 948 F.2d 1265, 1268-69 (Fed. Cir. 1991) (quoting *In re Oelrich,* 666 F.2d 578,581 (C.C.P.A. 1981)).

In *Glaxo Inc.* v. *Novopharm Ltd.,* 52 F.3d 1043 (Fed. Cir. 1995), for example the Federal Circuit relied on *Continental Can* in upholding claims to "Form 2" rantidine hydrochoride crystals based on conflicting evidence regarding the form produced in a prior-art process. The alleged infringer, Glaxo, presented evidence that the prior-art process produced Form 2 each of the 13 times its experts performed the process, whereas the patentee, Novopharm, presented evidence that only Form 1 was produced. *Id.* at 1047. The district court determined that the process could produce either form and therefore found that Glaxo had failed to establish that prior-art process inherently produced Form 2. The Federal Circuit held that the "district court correctly rejected Glaxo's anticipation defense." *Id.*

The Board of Patent Appeals and Interferences has also followed the rule that inherency may not be established by possibilities. In *Ex Parte Levy,* for example, the Board reversed a § 102 rejection of claims reciting a biaxially oriented polymeric balloon because the evidence of record regarding this feature was in conflict. The Board stated:

> According to the membrane equation calculations reported in Levy's declaration. . . , Schjeldahl's balloon could not possibly exhibit the tensile characteristics of a biaxially oriented balloon. Levy's calculations are inconsistent with those of Pinchuk . . . suffice it to say, ***the conflicting calculations taint the factual determination of inherency with impermissible conjecture.***
>
> *Ex parte Levy,* at 1464 (emphasis added)

Thus, Federal Circuit and Board precedent makes it clear that, to overcome a § 102 rejection based on inherency, Applicant need only demonstrate that the allegedly inherent feature does not *necessarily* flow from the teachings of the prior art.

Annealing-based recombination reactions can work, for example, by fragmenting two nucleic acid molecules that contain homologous but distinct nucleotide sequences, denaturing the fragments, annealing the fragments at points of homology, and extending the annealed fragments with a polymerase. The possible progeny sequences that could result from such a reaction are limited by the parental nucleic acid fragments that are put into the reaction. Whether or not the method proposed by Ahmann *could* result in a variant with a substituted amino acid sequence in, for example, the $ADX^{8}TIX^{9}EE$ segment, depends on the parental sequences used in the reaction. Ahmann states "[w]e will...attempt several different shuffles, with the most conservative including only algal Fe-hydrogenases and the least conservative incorporating up to twenty or more diverse Fe-hydrogenase parents simultaneously." (Ahmann, p.14, first full paragraph). In a "conservative" shuffle using only algal Fe-hydrogenases there is no possibility of obtaining a variant with a substitution in the $ADX^{8}TIX^{9}EE$ segment. Both *C. reinhardtii* hydrogenases (hyd1 and hyd2 in Table 1 of Ahmann, also listed as SEQ ID NOs: 24 and 26 in the instant application), which are algal, and the only other algal hydrogenase mentioned in Ahmann (hydA from *S. obliquus* in Table 1 of Ahmann, also listed as SEQ ID NO: 27 in the instant application) contain the identical amino acid segment ADLTIMEE. The most "conservative" shuffle proposed by Ahmann therefore has no chance of producing a variant with a substituted amino acid sequence in the $ADX^{8}TIX^{9}EE$ segment. No annealing-based recombination event between hydrogenases that have an identical sequence of ADLTIMEE can result in a variant with a different amino acid sequence at this segment.

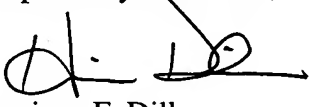
The claimed methods relate to rational, targeted mutagenesis of a previously undefined active site segment in an iron hydrogenase. Ahmann, by contrast, teaches a random mutagenesis method, namely DNA shuffling with full length, wild-type cDNAs. Nothing in Ahmann teaches "providing a mutagenized nucleic acid sequence derived from a first gene that encodes an iron-hydrogenase protein, wherein at least one amino acid from the segment $X^{1}X^{2}X^{3}FX^{4}X^{5}X^{6}GGVMEAAX^{7}R$ and at least one amino acid from the segment $ADX^{8}TIX^{9}EE$

are both substituted by a different amino acid in the iron-hydrogenase to generate the mutagenized nucleic acid sequence." Ahmann fails to explicitly or inherently anticipate the pending claims as amended. Withdrawal of the § 102 rejection is therefore respectfully requested.

No cancellation or amendment of any claim should be construed as an admission that the unamended claim does not constitute patentable subject matter.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-780-4777.

Respectfully submitted,



Harrison F. Dillon
Reg. No. 45,661

Solazyme, Inc.
Edison Technology Park
3475-T Edison Way
Menlo Park, CA 94025
Tel: 650-780-4777
Fax: 650-989-6700

Attachments: Paper copy of amended sequence listing
CD copy of amended sequence listing (2)